A quick start guide to developing continuous cell lines for cultivated seafood

This summary focuses on practical guidance for cell culture practitioners, containing:

- A "quick start guide" to developing a cell isolation protocol, focusing on the order of operations for optimizing different variables.
- Key technical considerations related to methodology, with specific recommendations where appropriate.

For additional context on how these recommendations were developed, please see the full-length guide. This document is an excerpt from that guide. The guidance provided here is intended as a starting point only, and results may vary according to species, cell type, or the handler.

A quick start guide to seafood cell line development

Providing a full protocol for seafood cell line development is beyond the scope of this report. Given the differences in needs for media and growth conditions between species, protocols will need to be determined through trial and error to a large extent. In addition, there is still a lot that we simply don't know.

While keeping those limitations in mind, this "quick start guide" represents our best attempt at outlining a series of general steps one could follow in developing a protocol. Our aim is to help you to avoid, identify, and troubleshoot some of the most common problems.

These steps (summarized in figure 1) are written with the goal of making things as easy as possible for someone who is new to this work. More experienced researchers may choose to take on bigger challenges or follow a different path from what we describe here.

These recommendations are intended to apply to both fish and crustaceans, unless otherwise stated. However, please note that we were able to compile more information on fish than on crustaceans, so our level of confidence in these recommendations is higher when it comes to fish.

1. Choose a species to work with, erring on the side of a species that is likely to be easy to work with. This is somewhat difficult to predict ahead of time, but we have included some general guidance below. Consider the animal's habitat and physiological context (e.g., the temperature, pH and osmolality of its aquatic environment), how easy it will be to access the tissues (assume you will need to do multiple isolations), and to what extent prior literature and tools—such as annotated genome sequences—are available. If you choose to work on crustaceans, you should be prepared for the fact that they are very likely to present additional challenges.
2. Choose the tissue you will work with and the cell type you will target. You may want to isolate a few tissues from the same animal. However, try not to go overboard in sampling too many tissues as this can add complexity and increase the risk of contamination or tissue degradation.
3. For your first experiment, focus primarily on testing a few combinations of methods for decontaminating the tissue. Pick an isolation method (explant or dissociation) to start with—you can optimize this later. We also recommend including antibiotics and antifungals in the media at this stage.

Which ones and at what concentrations can be adjusted experimentally, and these components can be removed in later passages once a contaminant-free culture has been established.

4. Once you are able to get contaminant-free cultures, begin systematically testing other parts of the isolation procedure. Try explant cultures as well as a few different enzyme types, concentrations, and exposure times to dissociate the tissue.
5. Next (or in parallel), test a few different media formulations, using prior cell culture literature on your chosen species (or close relatives) as a starting point. Multiwell plates can make this a lot easier. Also consider adjusting various other aspects of the culture environment, such as temperature, CO_2 concentration, humidity, and substrate choice.
6. The next obstacle you are likely to encounter is slow cell growth. Be patient with the cells, as they may simply need some time to adapt to the culture conditions, and test multiple combinations of variables to find what works best. Try to be organized in documenting these early experiments, but balance the need to observe the cells with the need to avoid excessive handling.
7. When cells begin to approach confluence and are ready to be passaged, be prepared that you may need to test a few sets of conditions for passaging. Altering the concentration of dissociation reagent (e.g., trypsin and EDTA), as well as the exposure time, can be important to get effective dissociation without damaging the cells. You may lose a few cultures to troubleshooting your passaging protocol and split ratio.
8. Once you have managed to successfully passage the cells a few times, they're growing well (this may require further optimization), and they're contaminant-free, congratulations! You've hit a key milestone. There's still much more to be done, but this is the point where, at least for fish, your chances of ending up with a successful cell line from a given isolation go from quite low to pretty good. Continue to maintain a few different cultures from this point forward if possible, as this will increase your overall chance of success if something goes wrong with one.
9. This is the point where you should start thinking about some early characterization steps to make sure the cells you're growing are the ones you want. At a minimum, make sure to test any promising cultures to make sure they are the species you think they are! Other characterization steps that are helpful at this point would be karyotyping (to allow for comparison with later-passage cells), differentiation capacity, and mycoplasma testing.
10. Be vigilant throughout the process for any changes in morphology or doubling time that could indicate a crisis event or senescence. If the cells do start to show signs that look like senescence (e.g., a flattened, enlarged appearance), be patient—they may recover with time.
11. Defining when a cell line has become immortalized can be a challenge, and there is no consensus among labs as to what an appropriate threshold is. Generally, between 50 and 100 doublings are reasonable thresholds. The presence of a clear crisis event seems to be the exception rather than the rule for fish cells, so this can provide evidence of immortalization in some cases but cannot be relied upon. Molecular markers, such as an upregulation of cell cycle activators and stable telomere length, can also provide helpful supporting evidence.
12. Once you are confident in the immortalization status of your cells, perform a thorough characterization prior to banking the cells, and confirm that they can be successfully frozen and thawed. If you still have multiple cultures going, you can compare them on key metrics like doubling time, metabolic efficiency, gene expression, and how well they respond to differentiation protocols. Be sure to document the conditions needed for growth of the cells in as much detail as possible to improve reproducibility across labs.

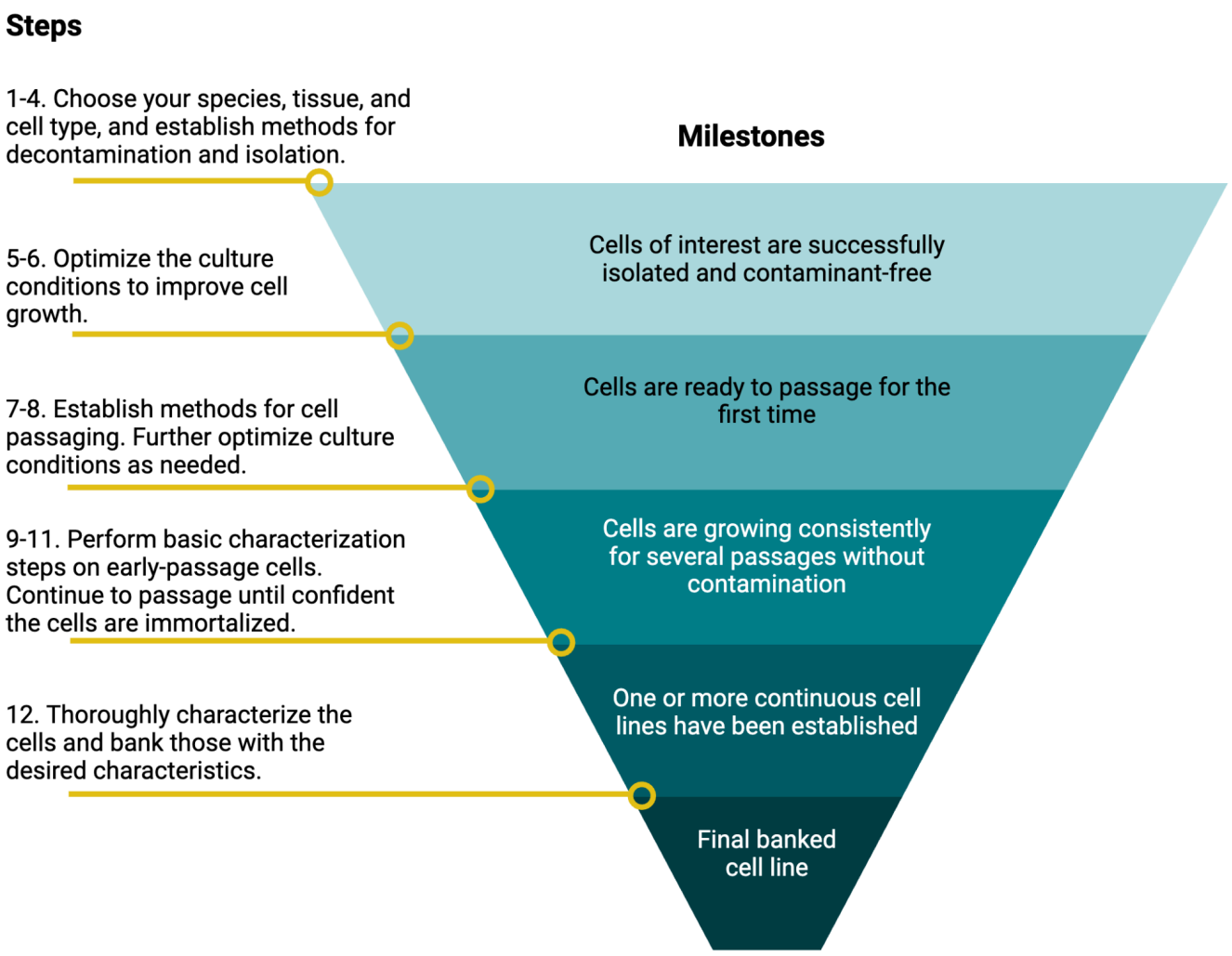

Figure 1. A visual summary of the steps described in the quick start guide. Created using Biorender.com.

Key technical considerations

Working within the framework described above, there are a number of decisions that will need to be made as part of the cell line development process. Below, we make some recommendations of either specific techniques or how to approach the decision of choosing a technique. These are primarily based on survey responses and interviews with researchers, supplemented with information from the published literature.

We recommend using this list in conjunction with the recommendations provided by Solhaug et al. (2025) and the methods described in primary research articles. We have compiled a list of relevant research papers (this includes those where only primary cells were isolated, but which are still likely to be useful as a reference for identifying isolation and culture conditions). For those isolating cells from crustaceans, Table 1 from Musgrove et al. (2024) is also a useful reference.

Much of what is discussed here is likely to be relevant to some extent across species. Points that are highly specific to the following are indicated as such:

fish

crustaceans

Spontaneous immortalization versus engineering-based approaches

There are two main approaches to producing a continuous cell line. The first is spontaneous immortalization, in which cells are repeatedly passaged until a stable proliferative population emerges. The second is to deliberately engineer a population of primary cells by introducing genes such as telomerase to induce continuous cell growth.

Generally, we would advocate for attempting spontaneous immortalization first when working with fish cells. Fish cells are commonly understood to be much more prone to spontaneous immortalization than those from terrestrial animals (Klapper, Heidorn, et al., 1998). Consistent with this, the challenges we heard about from the researchers we interviewed generally did not result from the cells' failure to immortalize, but were more often upstream problems related to cell isolation and maintenance. Engineering approaches can provide a useful backup option, and may introduce some other attractive opportunities (Riquelme-Guzmán et al., 2024), but in most cases, they are probably not needed to produce a continuous cell line. In the full-length version of this guide, we discuss additional details related to cell line engineering, including a case study on the use of engineering for immortalization of mackerel cells.

It is difficult to make a strong recommendation one way or the other when it comes to crustacean cells. Cell isolation and maintenance are especially challenging for these species, which makes it difficult to assess the likelihood of spontaneous immortalization. In theory, the fact that crustaceans express telomerase throughout life should point to a propensity for spontaneous immortalization as in fish (Klapper, Kühne, et al., 1998). However, how this translates to actual performance in cell cultures remains unclear (Musgrove et al., 2024). Establishing robust procedures for isolating and maintaining cells is a good goal to start with and is a necessary prerequisite for either approach.

Common pitfalls

According to our conversations with researchers, the problem that most commonly causes aquatic animal cell isolation experiments to fail is contamination, often thought to originate from the source tissue. This is usually the major hurdle for researchers new to isolating cells from these animals, but it is feasible to develop protocols that reduce contamination rates to a low level.

The second most common issue—and the most common for many of those who have successfully lowered their contamination rates—is slow cell growth that never picks up. It is not always clear whether this relates to the cell population itself or improper growth conditions. Both contamination and slow growth are common in fish and crustaceans, but more severe and prevalent in crustaceans.

While problems such as bacterial or fungal contamination and slow cell growth are easy to spot, other issues only become apparent when the cell line is deliberately characterized. Thus, it is possible to spend months maintaining a cell line only to find out that the cells are either of limited utility or entirely unusable. The version of this issue we heard about most often was species misidentification, often in the form of eukaryotic or other large-sized contaminants that were visually similar to the crustacean cells the researchers were looking for. To minimize the time lost to this issue, we strongly recommend performing some level of characterization (please see the section on "Best practices for cell line characterization" in the full-length guide) during early passages, including species identification.

Fortunately, almost all the other descriptions of culture failure we heard from researchers were those that occurred in the first few passages after cell isolation. Thus, as long as one is cognizant of the need for early characterization, it is usually possible to "fail fast" in these experiments and to avoid investing too much time in a culture that will ultimately not turn into a cell line.

Considerations for species and cell type selection

- Cells from warm water fish may be easier to work with than those from cold water fish.
- Having a fully annotated genome is very helpful for characterizing your cell line. Consider this when choosing a species to focus on (genomes can be searched on NCBI).
- According to a couple of researchers who have worked with multiple fish cell types, myogenic cells seem to be fairly intermediate in terms of the ease of establishing cell cultures and achieving immortalization. They are more difficult than fin, brain, spleen, and hard mesenchymal tissues such as bone, but are also not the most difficult to work with.
- If your primary cell type of interest is difficult to isolate and culture, one researcher recommended performing some cursory media optimization on a less-preferred but easier to culture cell type such as fibroblasts. The resulting formulation is likely to translate well to other cell types from the same species, making future isolation experiments on the target cell type much easier.
- Even within closely related species, there can be substantial differences in the ease of establishing continuous cell lines. For example, one researcher mentioned that trout cells are much easier to immortalize than Atlantic salmon. A couple of others mentioned salmon as being relatively easy to establish cell lines from, whereas another mentioned having particular trouble with salmon. Although this is extremely anecdotal, it is worth noting that the two researchers who characterized salmon as a difficult species worked primarily with Atlantic salmon, and the two who characterized it as easier worked with other species. Our very tentative recommendation would be to begin with genus *Oncorhynchus* rather than genus *Salmo* when developing cell lines from salmonids. However, please keep in mind that this is based on anecdotal evidence from only four researchers, so it is unclear if a true difference exists.
- To increase the applicability of your research to real-world problems, also consider the commercial relevance of your chosen species. Ideally, you would choose a species that is likely to be easy to work with that also has at least moderate commercial relevance.

Tissue sourcing and cell isolation

- Freshness of the tissue is important. If fish are killed rather than taken for a biopsy, it's important to consider whether the method will impact the viability of the tissue.
- Generally, younger animals are preferred. However, successful isolations from adult animals have been reported, and isolating from smaller animals can make it challenging to get a sufficiently-sized sample.
- Fish tissue is much more delicate than mammalian muscle, which makes using a scalpel to take samples difficult. One researcher recommended getting a chef's knife and cutting board to use for tissue sampling (autoclaved prior to use). Having a larger cutting surface makes it easier to avoid having the tissue fold over.
- Test a variety of isolation methods, including explants and enzymatic methods using a variety of enzymes, concentrations, and treatment times. Three of the researchers we spoke to reported having higher success rates with explants as opposed to enzymatic methods (this was mentioned twice spontaneously during the interview phase, and once in response to a direct written question while soliciting feedback on a draft of this report). This is fairly anecdotal evidence, but if one is limited on the number of experiments that can be performed, it might be preferable to start with explants over enzymatic digestion. Commercially-available isolation kits designed for aquatic species are beginning to emerge,

highlighting the value of B2B offerings in accelerating progress in cultivated seafood.

- Keeping the volume of culture media as low as possible can be helpful when establishing fish cell cultures. It's possible that this helps by encouraging fish cells to sit closer to the culture surface and therefore adhere better, or that it increases the concentration of helpful secreted factors. Changing only part of the media during the first few passages can also be helpful for this latter reason.
- It is possible to isolate directly into serum-free media, though this of course depends on already having established a workflow for cell isolation and a media formulation that works for a given species.
- Protocols developed in mammalian species can be a helpful starting point, but you should expect to need to do some optimization.
- Even when isolating cells from the same animal, different populations may show differences in morphology, gene expression, and doubling time. It's a good idea to keep multiple cultures going in parallel so you can pick the one that best suits your needs for future experiments.

The goal for cultivated seafood cell line development is generally not simply to develop a cell line, but to develop a cell line of the correct type and with certain desirable characteristics. Unfortunately, the use of advanced cell sorting techniques is limited for fish because of the dearth of appropriate antibodies, so fish cell cultures often represent a mix of cell types, or simply the cell type that grows best under the specified conditions (Solhaug et al., 2025). The situation is likely no better in crustaceans. As discussed below, single-cell cloning is rarely successful in fish cells, but when it is, it offers the opportunity for a defined and homogenous cell population (Ikeda et al., 2024). A more common technique that does not result in a homogenous population is to use some version of the pre-plating technique to select cells based on how readily they adhere to the culture dish. By separating the cells that readily adhere from those that are slower to adhere, it may be possible to achieve populations that are relatively enriched in fibroblasts or myoblasts, respectively (Alexander et al., 2011; Kim et al., 2022). This step does not need to be carried out during the initial cell isolation step, but rather can be used later once the cells are able to be trypsinized to select for certain cell populations (Y. Li et al., 2025).

In cases where it is feasible, we also recommend maintaining documentation of the health status of the donor animal, which may be important if you decide to commercialize the cell line down the road. For an example of what this documentation might look like, please see the dossier submitted by Wildtype to the U.S. Food and Drug Administration (FDA) (page 5). As discussed below, multiple cell isolations may be needed, especially for those new to this research, so this may be less necessary for initial experiments aimed at simply establishing procedures.

Testing and monitoring during cell line establishment

- Early testing for species identification can prevent excessive time spent on culturing the wrong cells. Suppliers can sometimes unknowingly ship animals of the wrong species, and contaminants can masquerade as the cells you want, especially when you're starting to work with an unfamiliar species or cell type. We heard about more instances of this with crustaceans than with fish.
- Take pictures of every passage and record doubling times. Subtle changes in morphology or growth rates might not otherwise be obvious, especially if you're working on multiple cultures at the same time, and can be important clues as to what's going on with your cells.
- Every time you check on the cells, you're potentially disturbing them and exposing them to light. For slow-growing cultures, sometimes it's better to leave them for some time and let them do their thing.

- Be very skeptical of any experiments using antibodies. Do positive and negative controls to make sure you're not seeing nonspecific staining, and if possible, complement these experiments with alternative methods like qPCR.
- Two respondents highlighted that senescence-associated β-galactosidase staining may not be a reliable indicator of senescence due to background staining and difficulties with quantification. Therefore, utilizing the absence of beta-gal staining alone as an indicator of immortalization is insufficient in fish cells. This was also highlighted by Solhaug et al. (2025).
- Off-the-shelf characterization tools are less available for aquatic species. It's likely worth it to spend the time upfront to build a characterization toolkit, learn to do your own karyotyping, etc.

Conditions for growth and passaging

- To the extent possible, try to screen for successful growth conditions early on in the process. One respondent listed this as a painful lesson they had learned, specifically with regard to media formulations. Others also indicated that they tend to do this sort of screening early on, with successful results. Systematic approaches like Design of Experiments (DoE) can be helpful, even before you have an established cell line, and multi-well plates with technical and biological triplicates are your friend.
- Dissociation can be hard on cells during early passages. Try to use the gentlest approach you can, and avoid excessive concentrations of both trypsin and EDTA. This was mentioned by several of the researchers we spoke to and has also been reported in the literature (N. Li et al., 2021). The exact concentration needed may depend on the cells in question, but for example, one researcher mentioned that 1 mM EDTA and 0.05–0.25% trypsin was effective. Alternative dissociation reagents, including the animal origin-free trypsin alternative TrypLE, as well as other formulations such as Accutase/Accumax, could also be worth testing.
- The use of enzyme-free, EDTA-based passaging methods have been successful for delicate human pluripotent stem cells, and may be worthwhile to attempt (Beers et al., 2012). Small molecules, such as Rho-kinase (ROCK) inhibitors, have also been reported in literature to boost survival of human pluripotent stem cells during passaging, and could be investigated for fish cell cultures.
- If working with cultures from multiple species, try to have a dedicated incubator and biosafety cabinet for each. This practice enables optimum culturing of cells that may need different conditions (temperature and CO_2), and also acts as an additional measure against cross-contamination.
- Even at later passages, fish myogenic cells can be fairly adaptable (within a range) to different temperature conditions. Depending on the species (and the media used), it may be possible to culture at room temperature without using an incubator.
- Even when a narrow temperature range is needed, it may be possible to use lower-cost, alternative equipment in place of an incubator. These may even be preferred over traditional cell culture incubators, the temperature settings of which may not go low enough for some aquatic species. We spoke to researchers who had seen success using reptile incubators and sourdough proofing ovens.
- Small details like the brands of consumables used can make a difference to cell growth. This sensitivity to variations among brands was also noted by Solhaug et al. (2025).
- Avoid passaging cells at too low of a density. Three different researchers mentioned that paracrine factors or cell-cell contact can be important, and cells will stop growing if they become too sparse. One researcher estimated that 25% confluence was too low and 50% was good, while another recommended not going below 30–40%. Splitting cells at a ratio of 1:2 or 1:3 is recommended. For fast-growing cells,

higher split ratios (~1:5) and lower confluency may be better tolerated. The researchers whose comments are represented here work with a variety of species, including fresh, salt, warm, and cold water. Doszpoly et al. (2025) reported gradually increasing the split ratio from 1:2 to 1:6, perhaps indicating a greater sensitivity to paracrine factors in early-passage cells. The importance of split ratio was also highlighted by Solhaug et al. (2025).

- Single-cell cloning rarely works in fish, possibly for the same reasons mentioned in the point above. However, there are exceptions (Ikeda et al., 2024).
- A couple of researchers mentioned using fish serum instead of FBS, but with differing results. In one case, serum from adult fish improved growth rates, but in another case, the serum appeared to be toxic to the cells. While we do not expect fish or mammalian serum to be the best choice at commercial scales, identifying sources of serum that perform better can be helpful both in lab-scale experiments and for identification of key factors that can be included in serum alternatives.
- Media development is not a main focus of this report, but choosing the right media is critical to the success of the cell line development process. While this is true of cultivated meat in general, seafood cells may have unique requirements when it comes to variables like osmolality, pH, and temperature. For more specific discussion of media formulations for cultivated seafood, please see The Science of Cultivated Meat.
- We spoke to one researcher who mentioned seeing toxicity as a result of too much glucose in the media. It has been shown that fish cells can adapt their metabolism in the absence of glucose and glutamine (Chen et al., 2005), suggesting that lower-glucose conditions might be worthy of investigation.

Contamination

- Contamination is the biggest challenge you are likely to encounter when starting out, especially for crustaceans, but multiple respondents indicated that they've managed to get to a point where it's a rare occurrence. It is possible!
- Isolations from larvae can be particularly difficult because of contamination from gut bacteria. Outer tissues like skin are more of a challenge than inner tissues like muscle.
- Contamination tends to be more likely with wild-caught fish, though it is possible to get a handle on, especially if not working with especially contamination-prone tissues.
- Allowing wild-caught animals to acclimate for some period in the lab under clean conditions may reduce contamination rates. One researcher mentioned that they see no significant differences in contamination rates between wild-caught and farmed fish that have undergone this acclimation step.
- One person recommended using amphotericin during isolations, but avoiding its use later on as it can impact cell growth. Penicillin/streptomycin are helpful throughout the cell line development process.
- Decontaminating the tissue before starting is important. How aggressively participants reported needing to do this varied, from simply wiping down the skin with ethanol to soaking a piece of tissue in bleach for two minutes and then cutting out and using the non-bleached inner tissue. It's a good idea to try a few different strategies (ethanol, bleach, Virkon, hydrogen peroxide, potentially different lengths of time) until you find something that works reliably.
- If you're isolating multiple tissues, be aware that there may be a cost in terms of the length and complexity of the dissection procedure. It wasn't clear if there was a causal link here, but one person reported struggling with contamination early on but seeing few problems recently, without an obvious change in methodology that explained this.

This person mentioned that they had gone from dissecting multiple tissues in each experiment to just a few, thereby streamlining the process, and speculated that this could have contributed to the lower contamination rates.

- This is based on a fairly small number of data points, but it seems like crustaceans may be more prone to contamination with "obscure" organisms. This includes various protists as well as less-common bacterial species. Fish contaminants, on the other hand, tend to resemble those one might expect to encounter in a mammalian cell culture lab, such as bacteria (including mycoplasma) and fungi (including mold and yeast).
- One researcher mentioned seeing much lower contamination rates when cells were isolated from crustaceans during their moulting and breeding season compared to those isolated at other times of the year.
- Because microorganisms can live in the cuticle, it's important when trying to isolate cells from crustacean muscle tissue to be careful to dissect out the muscle tissue only. A clean dissection that avoids the surrounding tissues is more likely to result in a contaminant-free culture.
- Contamination is an especially common issue with invertebrate cultures, including contamination by thraustochytrids (Walsh et al., 2025). Cytochrome oxidase 1 (CO1) sequencing works well for real-time monitoring of cultures, but can fail to pick up on low levels of contamination and requires you to know ahead of time what contaminants you're looking for. It is well-suited for quickly assessing the presence or absence of the species of interest. 18S community analysis can be a useful complementary technique as it gives a more complete picture of the ratio of different species present in a culture, with the downside that it takes longer to perform and so is less suited for real-time surveillance (Walsh et al., 2025).

Quality control steps for the final cell line

Thoroughly characterizing the final cell line is a crucial step that will help ensure its utility for cultivated seafood research. We also strongly recommend characterizing any cell lines that are acquired from external sources, as mis-authentication is fairly common. More details on recommended characterization steps can be found in the section on "Best practices for cell line characterization" in the full-length version of this guide.

It is generally a good idea to maintain multiple cultures from the target species and cell type. This both mitigates against the risk of losing a single culture and, perhaps more importantly, allows for the selection of the cell line with the best characteristics (e.g., growth rate, metabolic efficiency, differentiation potential, sensory characteristics) following this final characterization step. Depending on how stringent your requirements are, a higher or lower number of separate lines should be maintained.

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Acknowledgements

We gratefully acknowledge the researchers who contributed to this report by sharing their knowledge and expertise through surveys, interviews, emails, or some combination thereof. We thank the following individuals, as well as the six others who contributed while wishing to remain anonymous:

- Dr. Yusuke Tsuruwaka, Cellevolt
- Dr. SangYoon Lee, CellQua
- Dr. Mukunda Goswami, ICAR-Central Institute of Fisheries Education, Mumbai
- Dr. Charli Kruse, Institute of Medical and Marine Biotechnology of the University of Lübeck
- Dr. Daisuke Ikeda, Kitasato University
- Dr. Dinesh Parate, Laurus Bio Pvt. Ltd.
- Dr. Cathy Walsh, Mote Marine Laboratory
- Ian Johnson, Pitt BioForge
- Gavril Chong, Plant and Food Research
- Dr. Joana T. Rosa, S2AQUAcoLAB
- Dr. Karthick Velu, Sathyabama Institute of Science and Technology
- Dr. Shigeki Sugii, SIFBI, Agency for Science, Technology and Research (A*STAR)
- Marcus Vinicius Telles Teixeira, Sustineri Piscis
- Dr. Camila Luna da Silva, Sustineri Piscis
- Michael Saad, Tufts University Center for Cellular Agriculture (TUCCA)
- Dr. Frederico Ferreira, Universidade de Lisboa
- Dr. Fabiana Fernandes Bressan, University of São Paulo
- Divya Avnoor, University of Washington
- Rikard Saqe, University of Waterloo
- Dr. Gaston Otarola, Wildtype
- Dr. Nguyen (Nathan) Vo, Wilfrid Laurier University

The authors would like to thank Dr. Amanda Bess for her masterful project management and for her help on numerous aspects of this project, as well as Dr. Elliot Swartz, Dr. Simone Costa, Eileen Pauels, and Madeline Cohen for editing and other input on this report and the design of the survey. We would like to thank Dr. Yeshi Liang (GFI Consultancy), Isabela de Oliveira Pereira (GFI Brazil), Dr. Eileen Pauels (GFI Europe), Chandana Tekkatte (GFI India), and Marissa Bronfman (Future Ocean Foods) for their help circulating the survey or otherwise gathering input from cultivated seafood companies and researchers. We are also grateful to those researchers who contributed to the early stages of this project by engaging in collaborative discussions and providing feedback on the project's direction, and those who provided feedback and suggestions on a previously-published draft of this guide.

Suggested citation: Bomkamp, C. and Ravikumar, M. *A guide to developing continuous cell lines for cultivated seafood.* Washington D.C.: Good Food Institute. 2026 https://doi.org/10.62468/lgfr5676

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